4-Aminobiphenyl Hemoglobin Adducts in Fetuses Exposed to the Tobacco Smoke Carcinogen In Utero

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Maternal-fetal exchange of a potent tobacco-related human carcinogen, 4-aminobiphenyl, was studied in smoking (n=14) and nonsmoking (n=38) pregnant women. *N*-Hydroxy-4-aminobiphenyl, the active metabolite of 4-aminobiphenyl, forms chemical addition products (adducts) with hemoglobin. Levels of 4-aminobiphenyl hemoglobin adducts were measured in maternal-fetal paired blood samples obtained from smoking and nonsmoking women during labor and delivery. Carcinogen-hemoglobin adducts were detected in all maternal and fetal blood samples. Levels of such adducts were significantly higher ($P<.001$) in maternal and fetal blood samples from smokers: the mean 4-aminobiphenyl hemoglobin adduct level was 92 ± 54 pg/g of hemoglobin in blood samples from fetuses of smokers , and 17 ± 13 pg/g of hemoglobin in blood samples from fetuses of nonsmokers; the mean maternal 4-aminobiphenyl hemoglobin adduct level was 183 ± 108 pg/g of hemoglobin in smokers, and 22 ± 8 pg/g of hemoglobin in nonsmokers. Fetal carcinogen-adduct levels were consistently lower than maternal levels: the mean maternal to fetal ratio was 2.4 ± 1.1 in smokers and 1.9 ± .98 in nonsmokers. Fetal 4-aminobiphenyl hemoglobin adduct levels were strongly associated (correlation coefficient [r^2] = .51, P = .002) with maternal 4-aminobiphenyl hemoglobin adduct levels when paired samples from smoking mothers were analyzed. A measure of third-trimester tobacco smoke exposure based on number of cigarettes smoked per day, amount of each cigarette smoked, and depth of inhalation was associated (r^2=.59, P=.029) with maternal 4-aminobiphenyl levels but not with fetal 4-aminobiphenyl levels. This study demonstrates that a potent tobacco-related carcinogen, 4-aminobiphenyl, or its active metabolite, *N*-hydroxy-4-aminobiphenyl, crosses the human placenta and binds to fetal hemoglobin in concentrations that are significantly higher in smokers than in nonsmokers. [J Natl Cancer Inst 83:274–280, 1991]

Tobacco smoke is one of the most prevalent sources of in utero exposure to toxic substances. Evidence from clinical and laboratory studies suggests that access of tobacco smoke carcinogens to the human fetus is highly probable, and that the potential for tobacco smoke-induced human transplacental carcinogenesis exists and merits serious attention (*1*). Results from human studies demonstrate that: tobacco smoke toxins readily cross the placental membrane (*2-8*); tobacco smoke induces placental and fetal enzyme systems that are capable of bioactivating procarcinogens to mutagens (*9-12*); maternal smoking is associated with DNA damage in the placenta (*13*); and exposure to tobacco smoke in utero might increase the risk of developing childhood and adult cancers (*14-16*). In laboratory studies, tobacco smoke-related carcinogens such as 4-aminobiphenyl (*17*), benzo(*a*)pyrene (*17*), and tobacco-specific nitrosamines (*18*) readily cross the placental membrane. Furthermore, transplacental carcinogenesis occurs in laboratory animals exposed to cigarette smoke condensate (*19*), diethylnitrosamine (20), 3-methylcholanthrene (*21*), tobacco-specific nitrosamines (*22*), and benzo(*a*)pyrene (*23*).

Recent advances in the quantitative analysis of chemical addition products (adducts) make it possible to study the association between tobacco smoke exposure and carcinogen-induced DNA damage in fetal tissues (*13,24*). Everson et al (*13*), using the ^{32}P-postlabeling assay, detected a total of seven different DNA adducts in human placental tissue; three of the adducts were found almost exclusively in smokers. Shamsuddin and Gan (*25*) demonstrated the presence of benzo(*a*)pyrene 7,8-diol 9,10-epoxide (BPDE)-DNA adducts in human placenta by using anti–BPDE-DNA antibody and light microscope immunochemistry. Manchester et al (*26*) recently measured BPDE-DNA adducts in human placenta using ^{32}P-postlabeling and immunoaffinity chromatography. Elevated levels of several

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tobacco smoke carcinogen-DNA and tobacco smoke-protein adducts have been measured in tissues of adult smokers: polycyclic aromatic hydrocarbon-DNA adducts in white blood cells (*27*); benzo(*a*)pyrene-DNA adducts in bronchial cells (*28,29*), peripheral blood leukocytes, and pulmonary alveolar macrophages (*29,30*); 4-aminobiphenyl hemoglobin adducts (*30,31*) and hydroxyethyl hemoglobin adducts (*32*) in red blood cells.

In this study, we investigated the relationship between maternal smoking and 4-aminobiphenyl hemoglobin adduct level in the fetus. The substance 4-aminobiphenyl, a tobacco-related aromatic amine, is a potent human bladder carcinogen present in mainstream (4 ng per cigarette) and sidestream (14 ng per cigarette) tobacco smoke (*33*). The carcinogenicity of 4-aminobiphenyl is believed to derive from hepatic *N*-oxidation to *N*-hydroxy-4-aminobiphenyl, and subsequent hydrolysis to yield an electrophilic nitrenium ion that binds to DNA. In the blood, free *N*-hydroxy-4-aminobiphenyl is oxidized further within the erythrocyte to 4-nitrosobiphenyl, which forms a covalent adduct with hemoglobin (*34*). Several studies have demonstrated proportionality between carcinogen binding to DNA and carcinogen binding to hemoglobin (*35-38*). Therefore, 4-aminobiphenyl hemoglobin adduct level has been used as a dosimeter for DNA damage in tissues of adult smokers. Tannenbaum and colleagues (31) have measured elevated mean levels of 4-aminobiphenyl hemoglobin adducts (154 pg/g of hemoglobin) in smokers and detectable levels (28 pg/g of hemoglobin) in nonsmokers. In a recently published study, levels of 4-aminobiphenyl hemoglobin adducts dropped dramatically (from 120 pg/g of hemoglobin to 34 pg/g of hemoglobin) in human subjects successfully completing a smoking withdrawal program (*39*).

The presence of significantly elevated levels of a potent tobacco-smoke carcinogen in the hemoglobin of adult smokers underscores the importance of studying the maternal-fetal exchange of tobacco-related carcinogens during pregnancy. In order to increase our understanding of the access of tobacco smoke carcinogens to the fetus, we have determined: (*a*) the relative levels of 4-aminobiphenyl hemoglobin adducts in the fetuses of smokers and nonsmokers; (*b*) the relationship between measures of maternal tobacco smoke exposure during pregnancy and fetal 4-aminobiphenyl hemoglobin adduct levels; and (*c*) the relationship between maternal and fetal levels of 4-aminobiphenyl hemoglobin adducts.

Subjects and Methods

Research Subjects

All nonsmoking women who attended the Medical Center of Central Massachusetts-Memorial Obstetrics Clinic during 1988 were invited to participate in the study. Women who had been ex-smokers for less than 6 months were excluded from the study. Research subjects completed a validated investigator-administered questionnaire and a 7-day diary designed to assess exposure to environmental tobacco smoke during the third trimester of pregnancy. The study participants were asked to wear a passive nicotine monitor during all waking hours for the week during which they recorded the 7-day diary. In order to measure changes in passive exposure to tobacco smoke throughout pregnancy, a sample group of the nonsmoking pregnant women ($n = 17$) was asked to wear the nicotine monitor and complete the daily diary during both the first and third trimester. The development and validation of the diary, questionnaire, and nicotine monitor have been described in detail in previous publications (*40-42*).

All actively smoking women in the 34th through 38th week of pregnancy who attended the Medical Center of Central Massachusetts-Memorial Obstetrics Clinic in 1989 were also invited to participate in the study. An investigator-administered questionnaire was completed in order to determine details of smoking practice throughout pregnancy. Research subjects were asked to describe current average number of cigarettes smoked per day, changes in amount smoked throughout pregnancy, level and duration of inhalation, amount of each cigarette smoked, brand(s) smoked, and frequency of leaving unfinished cigarettes burning in an ashtray. Each participant was asked to complete a daily diary for 7 days. The diary provided a record of the number of cigarettes smoked per day during a typical week.

Laboratory Analysis

Maternal blood samples (20 mL) were collected from smoking and nonsmoking mothers during admission for labor and delivery. Fetal blood samples (20 mL) were collected from the umbilical vein immediately after delivery. Samples were refrigerated immediately and shipped on ice to laboratories at the Massachusetts Institute of Technology within 48 hours of delivery. Analysis of blood samples for levels of 4-aminobiphenyl hemoglobin (4-ABP) adducts was essentially the same as that reported earlier (*31*), except that a different internal standard, hemoglobin containing a perdeuterated 4-ABP adduct, was introduced. Briefly, the packed red blood cells from 10 mL of blood were washed with saline solution and lysed with distilled water/toluene. The supernatant after centrifugation was dialyzed against distilled water and used directly for analysis. Hemoglobin content was determined by Drabkin's assay and the internal standard, a solution of hemoglobin previously adducted with *N*-hydroxy-4-aminobiphenyl-d_9 and containing 150 pg of hydrolyzable amine, was added. After 30 minutes at room temperature, sufficient 10 *M* NaOH was added to yield a 0.1 *M* in NaOH mixture. The amines were extracted with hexane after 1 hour and derivatized with pentafluoropropionic anhydride. The hexane solution was concentrated to 20 µL for analysis by capillary gas chromatography with negative chemical-ionization mass spectrometry. Selected ion monitoring for the derivatives of the amines can detect less than 10 pg of 4-aminobiphenyl adduct per 10 mL of blood. A test of the precision of the assay using the deuterated adduct standard gave a value of 4%.

Results

Fifty-three nonsmokers and 20 smokers were enrolled in the study. Maternal-fetal paired blood samples were obtained from 38 nonsmokers and 14 smokers. Incomplete samples (eg, maternal or fetal blood sample only) were collected from 4 nonsmokers and 3 smokers. 4-Aminobiphenyl hemoglobin adducts were detected in all maternal and fetal blood samples. The concentration of carcinogen-hemoglobin adducts in the cord blood

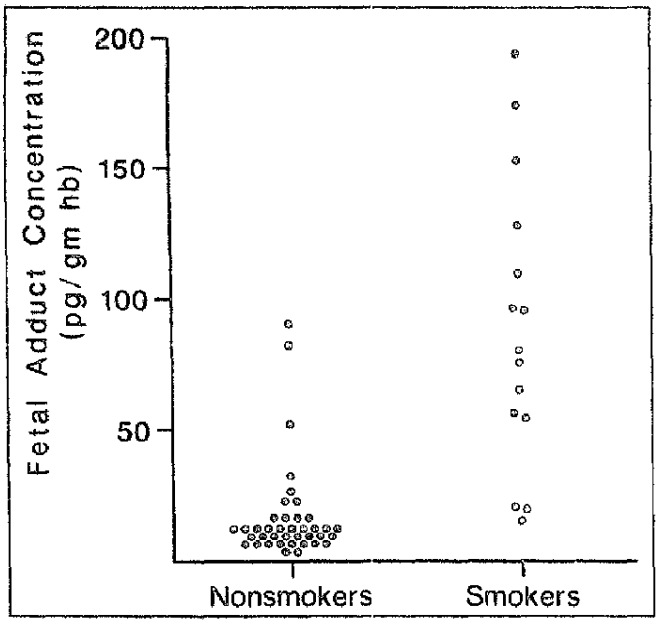

Fig 1. 4-Aminobiphenyl hemoglobin adduct concentration (pg/g of hemoglobin) in fetuses of nonsmoking (n = 40) and smoking (n = 16) mothers. hb = hemoglobin.

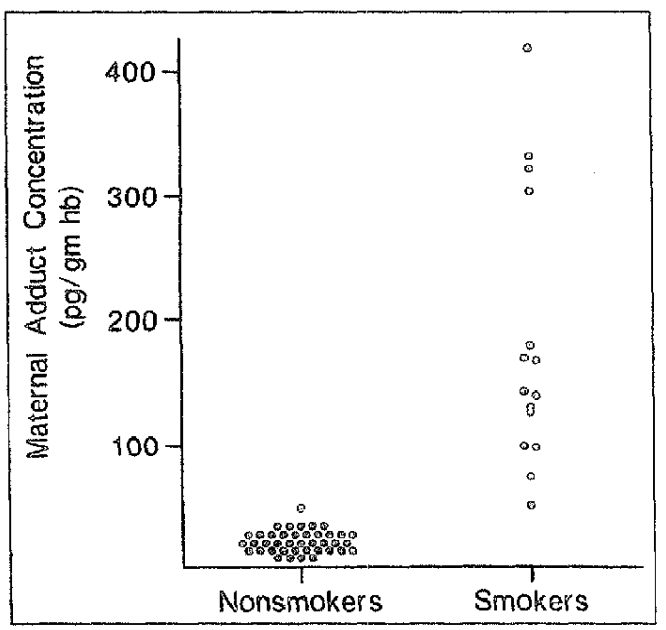

Fig 2. 4-Aminobiphenyl hemoglobin adduct concentration (pg/g of hemoglobin) in nonsmoking (n = 40) and smoking (n = 15) pregnant women. hb = hemoglobin.

of fetuses from smoking mothers (mean, 92 ± 54 pg/g of hemoglobin) was significantly higher ($P<.001$) than the concentration in cord blood from fetuses of nonsmoking mothers (mean, 17 ± 13 pg/g of hemoglobin). Carcinogen-adduct levels in maternal blood samples were significantly higher ($P<.001$) in smokers (mean, 183 ± 108 pg/g of hemoglobin) than in nonsmokers (mean, 22 ± 8 pg/g of hemoglobin). The levels of 4-aminobiphenyl hemoglobin adducts in maternal blood samples from smokers are in close agreement with levels reported in

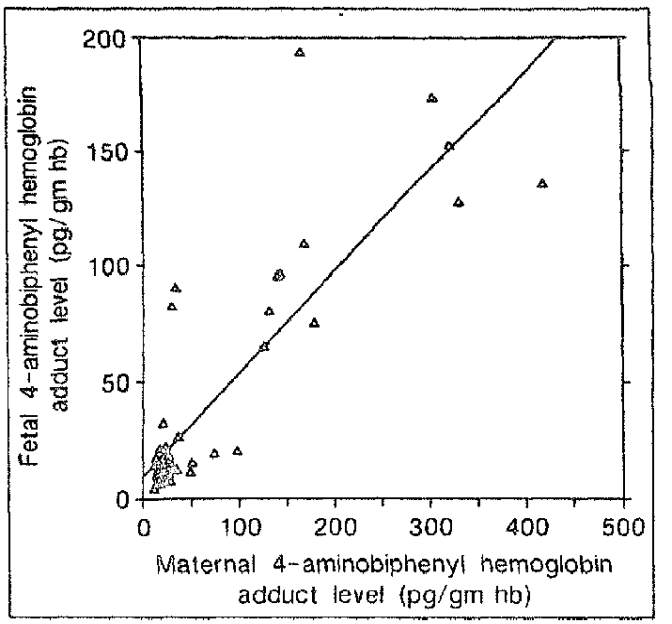

Fig 3. Simple linear regression of fetal 4-aminobiphenyl hemoglobin adduct concentration and maternal 4-aminobiphenyl hemoglobin adduct concentration in the total study sample (n = 52). hb = hemoglobin.

other studies (*31*,*39*). Maternal levels in nonsmokers are slightly lower than levels previously reported.

Fetal 4-aminobiphenyl hemoglobin adduct levels in smokers and nonsmokers were found to overlap (Fig 1). Three fetal blood samples from smokers fell within the range observed in nonsmoking adults (15,19, and 20 pg/g of hemoglobin), and three blood samples from fetuses of nonsmoking adults fell within the lowest range observed in smokers (52, 82, and 90 pg/g of hemoglobin). All maternal 4-aminobiphenyl hemoglobin adduct levels in nonsmokers were lower than the levels measured in smokers (Fig 2).

In order to characterize the maternal-fetal 4-aminobiphenyl hemoglobin adduct relationship, simple linear regression analysis was performed using maternal adduct level as the independent variable and fetal adduct level as the dependent variable (Fig 3). A significant correlation ($r^2 = .73$, $P<.001$) was found when the total study sample was included. To determine the extent to which the correlation is driven by the difference in smokers versus nonsmokers, separate linear regression analyses of the maternal-fetal 4-aminobiphenyl hemoglobin adduct concentrations in smokers and nonsmokers were conducted. Evidence for an association was stronger for smokers ($r^2 = .51$, $P = .002$) than nonsmokers ($r^2 = .10$, $P = .06$), (Figs 4 and 5).

Maternal 4-aminobiphenyl hemoglobin adduct levels were higher than fetal levels in 85% of the paired samples. In paired samples from nonsmokers, the ratio of maternal to fetal adduct level ranged from 0.4 to 4.4, with a mean value of 1.9 ± 0.98. In four paired samples from nonsmokers, maternal and fetal levels were equivalent; in three paired samples, fetal levels were higher. Maternal-to-fetal adduct ratios in smokers ranged from 0.9 to 4.9, with a mean value of 2.4 ± 1.1 (Fig 6). In one paired sample from a smoker, maternal and fetal adduct levels were equivalent; all other paired samples from smokers demonstrated adduct levels that were higher in the mother than in the fetus.

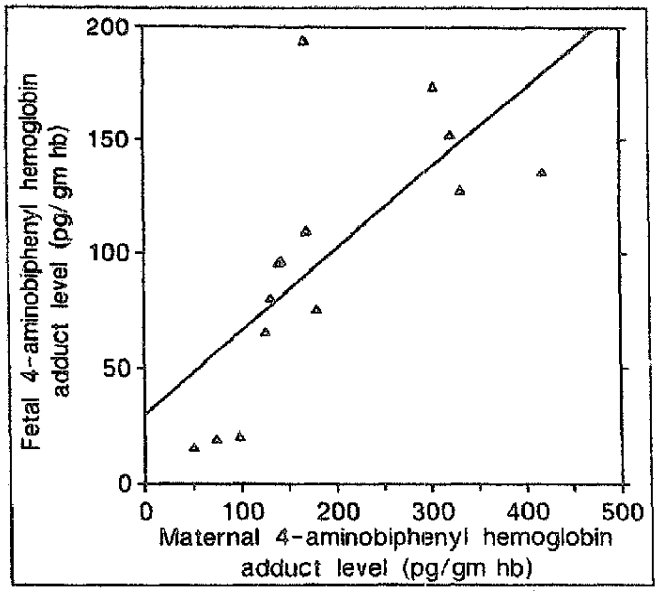

Fig 4. Simple linear regression of fetal 4-aminobiphenyl hemoglobin adduct concentration and maternal 4-aminobiphenyl hemoglobin adduct concentration in smokers (n = 14). hb = hemoglobin.

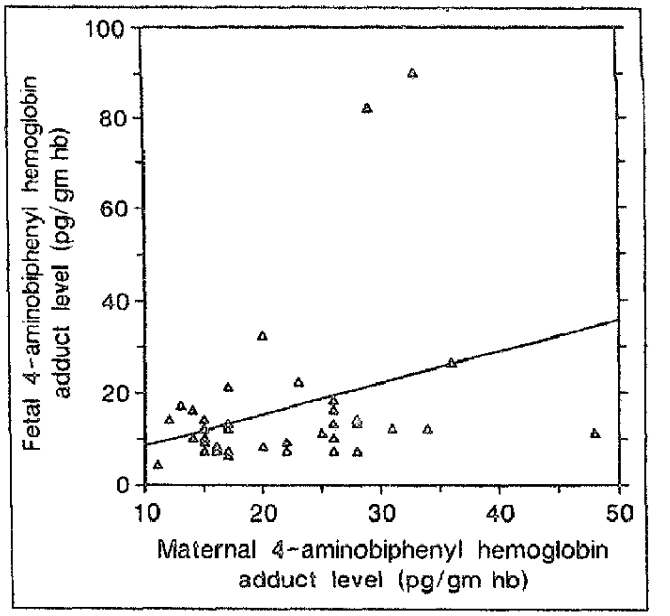

Fig 5. Simple linear regression of fetal 4-aminobiphenyl hemoglobin adduct concentration and maternal 4-aminobiphenyl hemoglobin adduct concentration in nonsmokers (n = 38). hb = hemoglobin.

To test for dose-response relationships, several measures of maternal tobacco smoke exposure were compared with maternal and fetal 4-aminobiphenyl hemoglobin adduct levels. For smokers, an estimate of the number of cigarettes smoked per day during the last trimester of pregnancy was derived from the questionnaire and daily diary. The estimate was based on the number of cigarettes smoked per day, the amount of each cigarette smoked, and the depth of inhalation. Maternal 4-aminobiphenyl hemoglobin adduct levels were positively associated with the exposure estimate (r^2 = .564, P = .029). Adduct levels in fetuses of smokers were not strongly predicted by the measures of maternal exposure (r^2 = .2, P = .08). For nonsmokers, the environmental tobacco smoke exposure score (*40*) derived from the questionnaire and diary, and the average concentration of nicotine calculated from the passive monitor, were compared with 4-aminobiphenyl hemoglobin adduct levels. Although maternal exposure to environmental tobacco smoke was not strongly associated with fetal adduct levels, evidence for a positive trend was observed when nicotine levels from passive monitors were compared with maternal 4-aminobiphenyl levels (manuscript in preparation).

Discussion

This study demonstrates that a potent tobacco-related carcinogen, 4-aminobiphenyl, or its active metabolite, N-hydroxy-4-aminobiphenyl, crosses the human placenta and binds to fetal hemoglobin. All fetal blood samples tested revealed detectable amounts of 4-aminobiphenyl hemoglobin adducts. Carcinogen-hemoglobin adduct levels in the fetuses of smoking mothers (mean, 92 pg/g of hemoglobin) were significantly higher than levels measured in the fetuses of nonsmokers (mean, 17 pg/g of hemoglobin).

Fetal 4-aminobiphenyl hemoglobin adduct levels were lower than maternal levels in 85% of the paired samples. The consistent step-down in fetal levels cannot be accounted for by decreased binding affinity of human fetal hemoglobin: in vitro measurements of adduct formation by reaction of *N*-hydroxy-4-ABP with fetal hemoglobin confirmed equivalent binding affinity of adult and fetal hemoglobin. The twofold difference in maternal and fetal levels observed in our study is consistent with animal studies of 4-aminobiphenyl hemoglobin transplacental

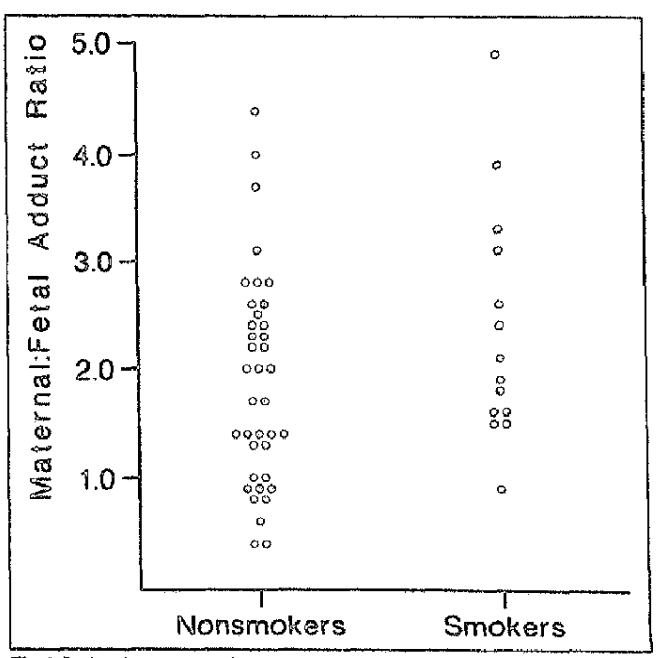

Fig 6. Ratio of maternal to fetal 4-aminobiphenyl hemoglobin adduct concentration in nonsmokers (n = 38) and smokers (n = 14).

transport. Lu et al (*17*) found detectable levels of 4-aminobiphenyl DNA adducts in all fetal tissues following maternal dosing in laboratory rats. Fetal levels were generally lower than maternal levels. Possible explanations for the lower fetal levels observed in our study include: immaturity of fetal enzyme-activating systems; placental trapping of active metabolites; carcinogen inactivation catalyzed by tobacco smoke-induced placental enzymes; and increased rate of degradation of the fetal 4-aminobiphenyl hemoglobin adduct. Fetal hemoglobin turns over at a significantly higher rate (lifetime = 65 days) than maternal hemoglobin (lifetime = 120 days). Therefore, if exposure to tobacco smoke decreases during the third trimester, relatively lower levels of carcinogen-hemoglobin adducts may be present in fetal blood samples obtained during delivery, secondary to increased degradation of the older, more heavily exposed red blood cells in the fetus. In our study, exposure to tobacco smoke may have decreased during the third trimester. Among smokers, 47% of the research subjects reported decreasing the number of cigarettes smoked per day during the final 2 months of pregnancy (mean decrease, 5 cigarettes per day). Among nonsmokers, 47% of the research subjects for whom first- and third-trimester nicotine levels were obtained demonstrated decreased nicotine levels during the third trimester (mean percent decrease, 58%).

The significantly elevated levels of 4-aminobiphenyl hemoglobin adducts in cord blood samples from smokers raises serious concerns regarding the potential for transplacental carcinogenesis. Although fetal levels in our study are consistently lower than maternal levels, studies of transplacental carcinogenesis in laboratory animals demonstrate that lower levels of carcinogens may initiate carcinogenesis when exposure occurs in utero. Administration of 60 mg of ethylnitrosourea per kilogram body weight to pregnant rats initiates 50 times as many tumors in offspring as would the same dose to adults (*43*). In addition, the observation that enzyme systems generally are activated earlier in human fetuses than in laboratory animals supports the possibility that activated tobacco smoke carcinogens may be present in fetal tissues during the processes of cell proliferation and differentiation (*44*). Carcinogen-hemoglobin adducts have been shown to be accurate dosimeters of DNA adduct formation in adult humans and laboratory animals (*35-38*). In the human fetus, however, DNA-repair enzyme activity is twofold to fivefold lower than in the adult (*45*). It is possible that DNA-repair activity in the fetus occurs at a slower rate and that DNA damage in the fetus is even greater than indicated by carcinogen-hemoglobin adduct levels.

The presence of elevated levels of a known human carcinogen in the fetal blood reinforces the need for further study of tobacco smoke-related transplacental carcinogenesis. Several epidemiologic studies have been conducted to look for a relationship between childhood and adult cancers and in utero exposure to tobacco smoke carcinogens. Stjernfeldt et al (*14*) reported a dose-response relationship between number of cigarettes smoked per day during pregnancy and cancer risk in offspring. The risk is doubled for non-Hodgkin's lymphoma, acute lymphoblastic leukemia, and Wilms' tumor. In a large prospective study, Neutel and Buck (*15*) found a nearly doubled incidence of leukemia in the offspring of mothers who smoked during pregnancy. Sandler et al (*16*) reported an increased adult risk (relative risk = 2.7) for hematopoietic malignancies related to gestational exposure to cigarette smoke. Significantly increased relative risk was found for Hodgkin's disease (relative risk = 4.4), non-Hodgkin's lymphoma (relative risk = 1.7), and acute leukemia (relative risk = 8.8). In a recent study, Janerich et al (*46*) reported that 17% of lung cancers among nonsmokers can be attributed to high levels of exposure to cigarette smoke during childhood and adolescence. Although these studies are hampered by the difficulty of separating in utero and childhood exposures, the results of our study support a transplacental relationship. The mean level of carcinogen-hemoglobin adducts present in the fetuses of smoking mothers in our study was four times higher than the mean level found in passively exposed nonsmoking adults. Assuming that children exposed to environmental tobacco smoke have carcinogen-adduct levels similar to those of passively exposed adults, in utero exposure represents a much more concentrated dose. In addition, in utero exposure may occur during a time of potentially increased vulnerability secondary to the rapid cell proliferation and differentiation in the developing fetus. In laboratory studies, Kauffman (*47*) demonstrated a close correlation between the number of proliferating epithelial cells and the number of tumors induced transplacentally by ethylnitrosourea at different gestational ages.

Several studies with animal models have demonstrated enhanced tumorigenesis in adult animals that were exposed in utero to carcinogens (*48,49*). Transplacental plus perinatal exposure to diethylnitrosamine more than doubled the frequency of incidence of tumors in adult animals when compared with either exposure by itself (*20*). These results underscore the need to look at adult as well as childhood cancers as possible outcomes of in utero exposure to tobacco-related carcinogens.

The failure to observe a stronger dose-response relationship between fetal carcinogen-adduct levels and maternal tobacco smoke exposure is not surprising given the complex genetic and environmental determinants of carcinogen metabolism. Butler et al (*50*) found a 44-fold variation in rates of 4-ABP N-oxidation in 22 liver microsomal preparations, and Cartwright et al (*51*) demonstrated a greater than 10-fold person-to-person variation in the activity of several enzymes involved with benzo(*a*)pyrene metabolism. Vineis et al (*52*) observed that levels of 4-aminobiphenyl hemoglobin adducts were higher in research subjects with genetically determined slow acetylation rates. Manchester and Jacoby (*9*) observed substantial overlap and variability of placental monooxygenase activity in research subjects within the same exposure groups (eg, nonsmokers, passive smokers, 1-20 cigarettes per day smokers, and >20 cigarettes per day smokers). In our study, overlap of 4-aminobiphenyl hemoglobin adduct concentration was observed in fetal blood samples from smokers and nonsmokers. Of particular concern are the elevated carcinogen-adduct levels measured in fetuses whose nonsmoking mothers demonstrated (*a*) minimal exposure to environmental tobacco smoke, and (*b*) maternal adduct levels within the lower ranges observed in nonsmokers. Information is lacking regarding the amounts and kinds of enzymes relevant to 4-aminobiphenyl metabolism in human fetal tissues, but interpersonal variations in such metabolism in placental and other fetal tissues may contribute to the complexity of predicting can-

cer burden. Pre-existing disease conditions or coexposure to environmental agents may further modify the genetically determined activity of the carcinogen.

In summary, this study confirms transplacental passage of a potent tobacco-related human carcinogen, 4-aminobiphenyl. The presence of significantly elevated levels of 4-aminobiphenyl hemoglobin adducts in the blood of fetuses from smoking mothers indicates that maternal smoking during pregnancy increases carcinogen-induced DNA damage in fetal tissues and may, therefore, be associated with increased risk of developing childhood and adult cancer. The presence of detectable and, in some cases, elevated carcinogen-adduct levels in the fetuses of passively exposed nonsmokers lends support to the hypothesis that maternal exposure to environmental tobacco smoke may also be associated with increased risk of developing cancer later in life. Future epidemiologic studies using recently developed techniques to estimate molecular dose will be required in order to test these hypotheses fully.

References

(1) EVERSON RB: Individuals transplacentally exposed to maternal smoking may be at increased cancer risk in adult life. Lancet 1:123–127, 1980

(2) MOCHIZUKI M, MARUO T, MASUKO K, ET AL: Effects of smoking on fetoplacental-maternal system during pregnancy. Am J Obstet Gynecol 149:413–420, 1984

(3) SMITH N, AUSTEN J, ROLLES CJ: Letter: Tertiary smoking by the fetus. Lancet 1:1252–1253, 1982

(4) SORSA M, HUSGAFVEL-PURSIAINEN K: Assessment of passive and transplacental exposure to tobacco smoke. *In* Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention (Bartsch H, Hemminki K, O'Neill IL, eds), vol 89. Lyon, France: IARC, 1988, pp 129–132

(5) VAN VUNAKIS H, LANGONE JJ, MILUNSKY A: Nicotine and cotinine in the amniotic fluid of smokers in the second trimester of pregnancy. Am J Obstet Gynecol 120:64–66, 1974

(6) ANDRESON BD, NG KJ, IAMS JD, ET AL: Letter: Cotinine in amniotic fluids from passive smokers. Lancet 1:791–792, 1982

(7) ETZEL RA, GREENBERG RA, HALEY NJ, ET AL: Urine cotinine excretion in neonates exposed to tobacco smoke products in utero. J Pediatr 107:146–148, 1985

(8) BOTTOMS SF, KUHNERT BR, KUHNERT PM, ET AL: Maternal passive smoking and fetal serum thiocyanate levels. Am J Obstet Gynecol 144:787–791, 1982

(9) MANCHESTER DK, JACOBY EH: Sensitivity of human placental monooxygenase activity to maternal smoking. Clin Pharmacol Ther 30:687–692, 1981

(10) VAUGHT JB, GURTOO HL, PARKER NB, ET AL: Effect of smoking on benzo(*a*)pyrene metabolism by human placental mirosomes. Cancer Res 39:3177–3183, 1979

(11) JUCHAU MR, NAMKUNG MJ, JONES AH, ET AL: Biotransformation and bioactivation of 7,12-dimethylbenz[*a*]anthracene in human fetal and placental tissues. Analyses of HPLC profiles and studies with *Salmonella typhimurium*. Drug Metab Dispos 6:273–281, 1978

(12) CONNEY AH: Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memorial Lecture. Cancer Res 42:4875–4917, 1982

(13) EVERSON RB, RANDERATH E, SANTELLA RM, ET AL: Quantitative associations between DNA damage in human placenta and maternal smoking and birth weight. J Natl Cancer Inst 80:567–576, 1988

(14) STIERNFELDT M, BERGLUND K, LINDSTEN J, ET AL: Maternal smoking during pregnancy and risk of childhood cancer. Lancet 1:1350–1352, 1986

(15) NEUTEL CI, BUCK C: Effect of smoking during pregnancy on the risk of cancer in children. J Natl Cancer Inst 47:59–63, 1971

(16) SANDLER DP, EVERSON RB, WILCOX AJ, ET AL: Cancer risk in adulthood from early life exposure to parents' smoking. Am J Public Health 75:487–492, 1985

(17) LU LJW, DISHER RM, REDDY MV, ET AL: ^{32}P-postlabeling assay in mice of transplacental DNA damage induced by the environmental carcinogens safrole, 4-aminobiphenyl and benzo(*a*)pyrene. Cancer Res 46:3046–3054, 1986

(18) CASTONGUAY A, TJALVE H, TRUSHIN N, ET AL: Perinatal metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in C57BL mice. JNCI 72:1117–1126, 1984

(19) NICOLOV IG, CHERNOZEMSKY IN: Tumors and hyperplastic lesions in Syrian hamsters following transplacental and neonatal treatment with cigarette smoke condensate. J Cancer Res Clin Oncol 94:249–256, 1979

(20) NAPALKOV NP: Some general considerations on the problem of transplacental carcinogenesis. *In* Transplacental Carcinogenesis (Tomatis L, Mohr U, eds), vol 4. Lyon, France: IARC, 1973, pp 1–13

(21) TURUSOV V, TOMATIS I, GUIBBERT D, ET AL: The effect of prenatal exposure of mice to methylcholanthrene combined with the neonatal administration of diethylnitrosamine. *In* Transplacental Carcinogenesis (Tomatis L, Mohr U, eds), vol 4. Lyon, France: IARC, 1973, pp 84–91

(22) CORREA E, JOSHI PA, CASTONGUAY A, ET AL: The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is an active transplacental carcinogen in Syrian golden hamsters. Cancer Res 50:3435–3438, 1990

(23) BULAY OM, WATTENBERG LW: Carcinogenic effects of subcutaneous administration of benzo(*a*)pyrene during pregnancy on the progeny. Proc Soc Exp Biol Med 135:84–86, 1970

(24) PERERA FP: The significance of DNA and protein adducts in human biomonitoring studies. Mutat Res 205:255–269, 1988

(25) SHAMSUDDIN AKM, GAN R: Immunocytochemical localization of benzo(*a*)pyrene-DNA adducts in human tissue. Hum Pathol 19:309–315, 1988

(26) MANCHESTER DK, WILSON VL, HSU I-C, ET AL: Synchronous fluorescence spectroscopic, immunoaffinity chromatographic and ^{32}P-postlabeling analysis of human placental DNA known to contain benzo(*a*)pyrene diol epoxide adducts. Carcinogenesis 7:2071–2075, 1986

(27) PHILLIPS DH, HEWER A, GROVER PL: Aromatic DNA adducts in human bone marrow and peripheral blood leukocytes. Carcinogenesis 7:2071–2075, 1986

(28) BAAN RA, VAN DEN BERG PTM, STEENWINKEL M-JST, ET AL: Detection of benzo(*a*)pyrene-DNA adducts in cultured cells treated with benzo(*a*)pyrene diol-epoxide by quantitative immunofluorescence microscopy and ^{32}P-postlabeling: Immunofluorescence analysis of benzo(*a*)pyrene-DNA adducts in bronchial cells from smoking individuals. *In* Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention (Bartsch H, Hemminki K, O'Neill IK, eds), vol 89. Lyon, France: IARC, 1988, pp 146–154

(29) VAHAKANGAS K, TRIVERS G, ROWE M, ET AL: Benzo(*a*)pyrene diolepoxide-DNA adducts detected by synchronous fluorescence spectrophotometry. Environ Health Perspect 62:101–104, 1985

(30) PERERA FP, SANTELLA RM, BRENNER D, ET AL: DNA adducts, protein adducts, and sister chromatid exchange in cigarette smokers and nonsmokers. JNCI 79:449–456, 1987

(31) BRYANT MS, SKIPPER PL, TANNENBAUM SR: Hemoglobin adducts of 4-aminobiphenyl in smokers and nonsmokers. Cancer Res 47:602–608, 1987

(32) TORNQVIST M, OSTERMAN-GOLKAR S, KAUTIAINEN A, ET AL: Tissue doses of ethylene oxide in cigarette smokers determined from adduct levels in hemoglobin. Carcinogenesis 7:1519–1521, 1986

(33) PATRIANAKOS C, HOFFMAN D: Chemical studies on tobacco smoke. LXIV: On the analysis of aromatic amine in cigarette smoke. J Anal Toxicol 3:150-154, 1979

(34) KADLUBAR FF, DOOLEY KL, BENSON RW, ET AL: Pharmacokinetic model of aromatic amine-induced urinary bladder carcinogenesis in beagle dogs administered 4-aminobiphenyl. *In* Carcinogenic and Mutagenic Responses to Aromatic Amines and Nitroarenes (King CM, Romano LJ, Schuetzle D, eds). Amsterdam: Elsevier, 1988, pp 173–180

(35) EHRENBERG L, MOUSTACCHI E, OSTERMAN-GOLKAR S, ET AL: International Commission for Protection Against Environmental Mutagens and Carcinogens. Dosimetry of genotoxic agents and dose-response relationships of their effects. Mutat Res 123:121–182, 1983

(36) NEUMANN H-G: Dose-response relationship in the primary lesion of strong electrophilic carcinogens. Arch Toxicol Suppl 3:69–77, 1980

(37) PEREIRA MA, LIN L-HC, CHANG LW: Dose-dependency of 2-acetylaminofluorene binding to liver DNA and hemoglobin in mice and rats. Toxicol Appl Pharmacol 60:472–478, 1981

(38) SHUGART L, KAO J: Examination of adduct formation in vivo in the mouse between benzo(*a*)pyrene and DNA of skin and hemoglobin of red blood cells. Environ Health Perspect 62:223–226, 1985

(39) MACLURE M, BRYANT MS, SKIPPER PL, ET AL: Decline of the hemoglobin adduct of 4-aminobiphenyl during withdrawal from smoking. Cancer Res 50:181–184, 1990

(40) COGHLIN J, HAMMOND SK, GANN PH: Development of epidemiologic tools for measuring environmental tobacco smoke exposure. Am J Epidemiol 130:696–704, 1989

(41) HAMMOND SK, LEADERER BP: A diffusion monitor to measure exposure to passive smoking. Environ Sci Technol 21:494–497, 1987
(42) HAMMOND SK, COGHLIN J, LEADERER BP: Field study of passive smoking exposure with passive sampler. *In* Indoor Air '87: Proceedings of the 4th International Conference on Indoor Air Quality and Climate, vol 2. Berlin: August 17-21, 1987, pp 131–136
(43) DRUCKREY H, PREUSSMAN R, IVANKOVIS S: N-Nitroso compounds in organotropic and transplacental carcinogenesis. Ann N Y Acad Sci 163:676–696, 1969
(44) LUCIER GW, LUI EMK, LAMARTINIERE CA: Metabolic activation/deactivation reactions during perinatal development. Environ Health Perspect 29:7–16, 1979
(45) MYRNES B, GIERCKSKY KE, KROKAN H: Interindividual variation in the activity of 06-methyl guanine-DNA methyltransferase and uracil-DNA glycosylase in human organs. Carcinogenesis 4:1565–1568, 1983
(46) JANERICH DT, THOMPSON WD, VARELA LR, ET AL: Lung cancer and exposure to tobacco smoke in the household. N Engl J Med 323:632–636, 1990
(47) KAUFFMAN SL: Susceptibility of fetal lung to transplacental 1-ethyl-1-nitrosurea: Its relation to epithelial proliferation. J Natl Cancer Inst 57:821–825, 1976
(48) VESSELINOVITCH SD: Comparative studies on perinatal carcinogenesis. *In* Transplacental Carcinogenesis (Tomatis L, Mohr U, eds), vol 4. Lyon, France: IARC, 1973, pp 14–22
(49) ARMUTH V, BERENBLUM I: Tritiated thymidine as a broad spectrum initiator in transplacental two-stage carcinogenesis, with phorbol as promoter. Int J Cancer 24:355–358, 1979
(50) BUTLER MA, GUENGERICH FP, KADLUBAR FF: Metabolic oxidation of the carcinogens 4-aminobiphenyl and 4,4′-methylene-*bis*(2-chloraniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. Cancer Res 49:25–31, 1989
(51) CARTWRIGHT RA, GLASHAN RW, ROGERS HJ, ET AL: Role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: A pharmacogenetic epidemiologic approach to bladder cancer. Lancet 2:842–848, 1982
(52) VINEIS P, CAPORASO N, TANNENBAUM SR, ET AL: The acetylation phenotype, carcinogen-hemoglobin adducts, and cigarette smoking. Cancer Res 50:3002–3004, 1990